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CeNeS Limited
Compass House
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Chivers Way
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Cambridge, CB4 4ZR

Patents ADP number *(if you know it)* 7313877002

If the applicant is a corporate body, give the country/state of incorporation — United Kingdom

4. Title of the invention

Interface Patch Clamping

5. Full name, address and postcode in the United Kingdom to which all correspondence relating to this form and translation should be sent

Reddie & Grose
16 Theobalds Road
LONDON
WC1X 8PL

Patents ADP number *(if you know it)* 91001

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Signature: J N Goodman Reddie & Grose

Date: 17 March 1999

12. Name and daytime telephone number of person to contact in the United Kingdom: S J N GOODMAN 01223-360350

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INTERFACE PATCH CLAMPING

Introduction

The present invention provides a novel development of the conventional patch clamp technique. This novel technique is referred to as the interface patch clamp method.

Voltage gated ion channels are potential targets for a considerable range of novel treatments in a variety of disease states. The development of the patch clamp technique has provided a powerful method for the study of ion channel function and pharmacology in whole cells. However, while the patch clamp technique provides a definitive method for the investigation and screening of drugs with potential activity on voltage gated ion channels, the technique is currently highly dependent on the skill of the operator and tends to be very slow for drug screening. The present invention provides a method for increasing the rate at which compounds may be screened for ion channel blocking/agonist activity using the patch clamp technique. The method can retain the essential features of the conventional patch clamp recording system while facilitating automation of the major time-consuming components of the technique.

Background: Conventional Patch Clamp

The success of the patch clamp technique is derived from the ability to form "tight" (i.e. high resistance: Giga Ohm) electrical seals between an area of the cell membrane (the Patch) and the tip of a pipette. The patch clamp pipette is usually made from glass. The formation of the G-seal is dependent on the profile of the top of the pipette, and is enhanced by the application of suction to

the interior of the pipette. The requirements for the formation of the G-seals are well established and the process is usually monitored electrically by display of the current pulse recorded in response to a small voltage step applied throughout seal formation. After formation of a G-seal, the area of membrane under the pipette may be disrupted to obtain whole cell voltage clamp recording mode.

The sequence of events leading to successful G-seal formation and whole cell recording mode using pre-formed patch pipettes is as follows:

1. Selection of a suitable cell.

2. The patch pipette is positioned approximately 50 microns above the cell.

3. The pipette is lowered until the cell surface is deformed by the pipette tip.

4. Negative pressure is applied to the interior of the pipette until a G-seal is formed between the pipette tip and the cell membrane.

5. Whole cell recording mode is established by the application of further negative pressure which disrupts the cell membrane in the area under the pipette tip.

Steps two and three are slow and require considerable manual dexterity and a high level of operator skill. Visualisation of the cells and the patch pipette requires the use of a high quality microscope and, in order to position the pipette, a high quality three axis

micromanipulator with sub-micron resolution in each axis is required.

Summary of the Invention

According to the invention, interface patching can utilise a patch pipette of conventional type. Cells are supported on a liquid/air interface at one end of a capillary tube (e.g. made of glass, polyethylene or other suitable material). The axis of the patch pipette is in line with the axis of the tube so that the pipette tip can be manipulated into the opening of the tube where the cells are supported at the air/liquid interface. The capillary tube or the patch pipette can be mounted onto a single axis manipulator. Only one manipulator is required and this may be used to move either the patch pipette or the capillary tube. Whole cell recording mode is established as follows:

6. A layer of cells is established at the interface between the extracellular physiological solution (the liquid in which the cells are suspended) and air by dipping the capillary tube into a suspension of cells. The density of cells in the suspension must be sufficient to provide a sufficient number of cells to form a layer of cells at the interface.

7. Electrical contact with the extracellular solution is established via a non-polarizable electrode (e.g. an Ag/AgCl wire) and the tube is mounted either to a fixed clamp or single axis manipulator.

8. A patch pipette is provided which can be filled with electrolyte solution.

9. The patch pipette is mounted concentrically with the capillary tube either via a single axis manipulator or fixed clamp (if the capillary tube is to be moved). The pipette filling solution is connected via the non-polarizable electrode to the headstage of a conventional patch clamp amplifier. The pipette holder allows suction to be applied to the pipette interior.

10. Cell attached patch mode of recording is established by bringing the pipette tip in contact with the interface by moving the pipette and the capillary tube respectively together along the single mounting axis (e.g. either by moving the pipette towards the tube and interface or vice versa). On entry into the interface the movement of the pipette and capillary tube together is stopped and the pipette current is offset to zero on the patch clamp amplifier. The resistance of the pipette increases when the pipette contacts one of the cells at the air/liquid interface. Suction is then applied to the interior of the pipette and the pipette and capillary tube are moved closer together until the pipette tip is located inside the capillary tube.

Initial seal formation between the pipette tip and the cell may also be assisted by the application of gentle suction during entry of the pipette into the interface.

A G-seal is formed between the patch pipette tip and the cell membrane by the application of further suction to the interior of the pipette and monitoring the pipette resistance.

11. Following the formation of cell attached patch mode, the suction is released, pipette current is offset to zero and a holding voltage applied to the pipette (e.g. -60mV).

12. A whole cell recording is obtained by the application of further suction to the pipette interior until the whole cell recording mode is established in conventional manner.

According to this invention it is preferred that the capillary tube should be mounted in an upright orientation (i.e. essentially vertically) with the air/liquid interface at the downward end of the tube.

This has the advantage that suspended cells will tend to "sediment" naturally to the downward end of the tube and be collected there in a layer. The layer will preferably be several cells deep and loosely packed. Thus according to the invention the pipette tip may be moved upwardly relative to the air/liquid interface at the tube end (either by moving the pipette or the tube along the single axis) so as to come into contact with a cell in the layer at the interface. The relative density or concentration of cells at the interface compared to the density in the bulk of the liquid in the tube ensures a high probability that a cell can be collected on the tip without the need for visualisation of the operation and without the need for multidirectional manipulation of the tip/cell positional relationship. Surprisingly it has been found that G-seal formation between the cell and the pipette can occur without pressing the cell against a solid substrate.

Where the arrangement is intended to operate with the pipette in an upright orientation (i.e. essentially

vertically) with the tip uppermost and pointing upwardly, the pipette should be constructed so as to prevent the filling electrolyte solution flowing out and being lost. This may be achieved for example by use of a custom-made mounting assembly and/or by shaping the pipette body to prevent loss of filling solution (e.g by bending the pipette shaft into a U- or J- shape).

In a further aspect defined in the appended claims the invention relates to control logic or a computer control process for controlling an apparatus or method according to the aspects of the invention set out above. The control logic, implemented as software stored in a computer or on a computer-readable medium, may advantageously control the one or more motors or micromanipulators of the apparatus, and other functions such as pipette pressure and the flow of perfusion solutions or the like, in response to electrical parameters of the apparatus such as pipette resistance. Thus, the method and apparatus may in a preferred embodiment may be substantially automated, in particular requiring no visual imaging of the cell. All control may advantageously be achieved via electrical signal sensing.

Further advantageously, the control logic may control a plurality of interface patch clamp apparatuses in a substantially fully automated manner, thus obtaining substantial increases in speed of operation compared with the prior art.

The invention is illustrated by way of example in the accompanying figures in which:

Figure 1a shows a capillary tube containing a suspension of cells;

Figure 1b shows the cells having formed a layer at the air/liquid interface at one end of the capillary tube;
Figure 2 shows a general arrangement of the interface patch clamp recording equipment with moveable capillary tube;
Figure 2a shows an Apparatus for Interface Patch Clamping with drug/compound application;
Figure 3 shows the cell attached to the patch pipette ready for recording mode.
Figure 4 shows drug/compound addition during interface patch clamp recording: start position;
Figure 5 shows drug/compound addition during interface patch clamp recording: extracellular solution added to dish and dish moved down;
Figure 6 shows drug/compound addition during interface patch clamp recording: solution in dish brought into contact with interface region;
Figure 7 shows drug/compound addition during interface patch clamp recording: capillary raised above surface of solution in dish;
Figure 8 is a flow diagram of control logic embodying a further aspect of the invention; and
Figure 9 is a flow diagram of an example of the G-seal formation steps of figure 8.

Referring to figure 1a; a capillary tube (1) of appropriate size can pick up and hold a liquid sample (2) containing cells (3) in suspension. The sample can be picked up simply by dipping the tube end into a suitable bulk liquid reservoir. The liquid in the tube forms an air/liquid interface (4) at the tube end (5). The cells are initially distributed throughout the liquid relatively evenly.

Referring to figure 1b; with the tube in an upright essentially vertical orientation, the cells tend to sediment and to pack loosely together at the lower end of the tube by the tube end to form a layer (6) several cells deep. It will be appreciated by those skilled in the art that the density and depth of the cell layer can be determined by such factors as the cell concentration in the original suspension, the sedimentation time, the relative density of the cells and the liquid etc. It will also be appreciated that means could be devised to encourage or assist cells to migrate from the liquid towards the air/liquid interface rather than or as well as relying on gravitational sedimentation alone. The figure also shows the top of a patch pipette 8 pointing upwardly towards the interface.

Referring to figure 2; an arrangement is shown in which a single axis manipulator is used to move a capillary tube (1) held in a clamp (7) relative to a fixed patch pipette (8) held in a clamp (9). It will be apparent to those skilled in the art that this could be reversed so that the pipette is moved and the tube is fixed. The figure shows the tube clamped in a linear bearing sliding block (10) attached to a motorised single axis manipulator (11). The manipulator should be controlled preferably by computer in order to allow the motion of the manipulator to be varied by feedback from the patch clamp amplifier as described herein. The patch pipette is provided with a connection (12) to a conventional headstage. The system is also provided with a source of variable suction under the control of the patch clamp amplifier/computer.

In figure 2a an arrangement is shown in which additional electromechanical micromanipulators have been added. The micromanipulator labelled (13) is for moving the glass

capillary under automated or manual control. A second micromanipulator (14) moves the dish for drug application up and down the glass capillary. A third micromanipulator (15) moves a modified pipette holder to provide electrical contact with the pipette and a means of applying suction to the interior of the pipette. Rotational bases (16 and 17) allow the pipette holder to be moved in and out of the recording area and rotation of the pipette through 180 degrees for filling with pipette solution.

The figure also shows additional features, namely; a pipette holder (18); a patch clamp headstage (19); and a dish holder (20).

A version of the apparatus is envisaged in which patch pipettes will be loaded and filled automatically under software control. It is envisaged also that the loading of capillary glass into the apparatus and the filling with cell suspension will also be automated.

Referring to figure 3; a G-sealed cell 3 is shown held on the tip of the patch pipette 8 and positioned within the entrapped liquid volume in the tube.

Cell attached patch and/or whole cell (voltage clamp) recording may then be carried out.

The invention described herein has a number of significant features:

- Visualisation of the pipette and the cell is not required.
- Novel recording configuration that would not be considered as obvious.
- Surprisingly G-seal formation occurs without pressing the cell against a hard substrate.

- Cells form a layer at the solution-air interface.
- G-seal formation may be achieved using electronic feedback alone.
- There is no requirement for optical recognition/feedback.
- The system can be automated.
- Multiple recording capillaries and pipettes may be employed in order to allow recordings to be made simultaneously from many cells.

A method of operation of the apparatus of the embodiment under software control to achieve various of these advantages is described below.

In order to use the invention for screening a compound (e.g. for ion channel blocking/agonist activity) the compound of interest needs to be applied to the cell attached to the patch pipette. It will readily be appreciated that this could be achieved in different ways, for example by adding the compound to the extracellular liquid in the capillary tube either before or after G-seal formation. One additional advantage of the invention is that the liquid in the tube could be arranged in layers (e.g. containing different compounds or different concentrations of compounds) and the single axis manipulator could then be used to physically move and position a cell on a pipette tip into a chosen layer (e.g. by moving the G-sealed cell on the tip further up the tube away from the air/liquid interface at one tube end).

A further example of how the effects of compounds may be studied is illustrated in figures 4 to 7.

Figure 4 shows a capillary (1) containing the cell suspension (2) and patch pipette (8) in the recording

position for whole cell recording from a cell at the pipette tip. In addition, the capillary tube has been inserted through a hole (21) made in a dish (22) (e.g. 35mm plastic culture dish or similar). The dish is made of a material with hydrophobic properties and the hole allows the dish to be raised and lowered along the axis of the capillary by means of a micromanipulator (14).

Fig 5 shows the dish after it has been filled with extracellular physiological solution, which may contain the drug to be studied, or the drug may be at a later stage. Surprisingly, if the fluid level in the dish is low, leakage through the hole does not occur because the tendency to leak is counterbalanced by:

1. The surface tension of the water
2. The attraction of the water/solution to the glass capillary

After adding the solution to the dish, it is lowered in the direction of the arrow 2

Fig 6 shows the solution in the dish in contact with the end of the glass capillary and the patch pipette. The dish and the capillary are now raised simultaneously (arrows 1 and 2) in order to position the pipette tip/cell within the layer of liquid in the dish. If drug is present in the dish at this point and the capillary and dish were moved upwards rapidly, this would constitute a rapid application system particularly useful for the study of agonist responses that desensitise

Fig 7 shows the effect of raising the capillary so that it is not in contact with the liquid in the dish. The pipette tip/cell remains immersed in the external solution layer in the dish. The solution may be exchanged readily by perfusion of the dish and this allows multiple drug additions and dose response curves to be obtained while recording from the one cell.

It will be readily appreciated by those skilled in the art that:

1. The stability of recording using the interface patch clamp technique may be superior to that of conventional patch clamping. The greater stability of interface patch clamping is because the cell is held by the patch pipette alone. In conventional patch clamp recordings the cell is held by the patch pipette and a solid substrate and vibration tends to move the pipette relative to the substrate causing loss of the G-seal. The interface patch clamp is, in contrast to conventional patch clamp apparatus, relatively insensitive to vibration during drug application.
2. This method of drug application could be applied to a plurality of recording pipettes/capillaries and form the basis for a high throughput electrophysiological

assay system. It will readily be appreciated that the Interface Patch Clamp technique could be used with multiple pipettes and multiple capillaries in a manner in which each pipette enters its respective aligned capillary either individually in sequence or all together. Although not currently preferred, a single pipette could be used which is caused to enter more than one capillary sequentially. Multiple patch clamp recordings could be made either sequentially or simultaneously, depending on the application.

Control Logic for an Automated Patch Clamp System

Introduction

The following describes an embodiment of the control logic required to allow automation of a patch clamp system employing the Interface Patch Clamp technique described herein. The logic described will control one or more electromechanical micromanipulators/translators in order to patch clamp cells and apply drugs/compounds in order to screen for activity on membrane ion channels. A major advantage of the logic described is that automation is achieved in this system by the use of feedback from signals from the patch clamp amplifier and no image recognition software is required.

Methods

Inputs to the program are required from the patch clamp amplifier as follows:

Imon = current monitor output
Vhold = holding potential

Inputs to the program derived from patch clamp amplifier output signals are required as follows:

Inoise = base line current noise recorded from Imon
Rpip = pipette resistance
Rtot = Total resistance
Rs derived from Imon signal during voltage step

It is envisaged that these signals and evaluated values will be obtained from existing software via a suitable software interface. These signals and evaluated values are further defined in the list of variables and parameters below.

Inputs from manipulators/translators are required as follows, or from the following devices.

Patch module micromanipulator encoder
Capillary clamp/loader encoder and empty signal
Pipette automated clamp/loader encoder and empty signal
Two axis translator encoders for cell dipper
Drug application micromanipulator encoder
Pipette holder micromanipulator encoder

Control outputs from computer are required for the following devices.

Patch module manipulator
Pipette automated holder
Capillary loader/clamp
Pipette loader/clamp

Two axis translator for cell loading system
Pipette clamp
Suction device
Drug application manipulator
Drug perfusion solenoid valve system

The software uses signals derived from the patch clamp amplifier in order to control a number of peripheral devices which carry out patch clamping using the Interface Patch Clamp technique. The devices controlled by the logic comprise a number of micromanipulators, a suction device for the patch pipette and a valve system for perfusion of a recording chamber, such as the dish described above.

A number of parameters are given pre-set values which can be changed by the operator to suit different experimental conditions.

Summary of the control logic for the automated Interface Patch Clamp

Initial seal formation

The sequence of movements required for formation of a G-seal is unique for interface patch clamping and involves the control of at least one single axis manipulator (e.g. the patch module motor, although either the pipette or the capillary may moved to achieve the necessary relative movement between them) with feedback from the patch clamp amplifier. In a first embodiment of the control logic, the pipette is initially spaced from the capillary, as illustrated in figure 1b for example, and is moved towards the liquid/air interface at the capillary end until a change in the current monitor signal is recorded when the patch pipette enters the liquid/air interface and this

signal is used as the trigger to stop the micromanipulator. The pipette resistance may be derived from the output of the patch clamp amplifier and initial seal formation is monitored by recording the change in pipette resistence. If the resistance of the pipette does not increase beyond a pre-set value, the control logic infers that no G-seal has been formed and activates the patch module motor to move the liquid/air interface and the pipette apart until the resistance increases, which may occur when the pipette tip is withdrawn from the liquid or when a narrow neck of liquid is drawn out by surface tension between the pipette tip and the capillary end. When a resistance increase to a pre-set value is recorded suction is applied to the interior of the patch pipette and the patch pipette and the liquid/air interface are moved towards each other to a pre-set point, in a further attempt to form a G-seal with a cell.

Whole cell recording mode

After formation of the cell attached patch clamp recording mode, whole cell mode is obtained by the application of suction to the interior of the patch pipette while simultaneously monitoring the current (Imon) for capacitative transients. In the logic described, the formation of whole cell recoding mode is detected by a threshold crossing method but it will be apparent to those skilled in the art that other methods may also be employed e.g. online FFT (Fast Fourier Transform), Template Matching etc. The control logic checks for incorrect detection of whole cell mode before activating the experimental protocol.

Cell quality test

This routine monitors the quality of voltage clamp by comparing the series recorded resistance with a value related to pipette resistance. It will be appreciated that this method may be further enhanced by relating the acceptable series resistance to the amplitude of current evoked by a voltage step. In addition, an additional loop may be added to include the possibility of recording with a maintained level of suction applied to the pipette in cells that exhibit continuously increasing values of series resistance. The quality of the cell is also monitored by the holding current which should not be more negative than a pre-set value. It will be appreciated that this method could be enhanced by relating the acceptable value for holding current to the amplitude of the current in response to a voltage step.

Drug/compound application

The initial phase of drug application is unique to the interface patch clamp technique and involves the control of two single axis micromanipulators. The movements required utilise the position of the patch module micromanipulator recorded on entry into the interface as a reference point. After the cell has been immersed into solution contained in a perfusion chamber, the control logic calls a routine to carry out perfusion of the chamber via the activation of solenoid flow control valves.

Control Logic in detail

Variables/parameters

P = Pipette pressure relative to atmospheric pressure defined as 0
d = Patch module motor position
d0 = Patch module motor start position
d1 = Patch module motor position following entry of pipette into interface
d2 = Patch module motor position with pipette in recording position
d3 = Patch module motor position for chamber perfusion
dapp = Drug application module micromanipulator position
dapp0 = Drug application module micromanipulator position 0
dapp1 = drug application micromanipulator position increment 1 Pre-set increment
dapp2 = drug application micromanipulator position increment 2 Pre-set increment
Rs = Series resistance
Cslow = Slow capacitance compensation
Cfast = Fast capacitance compensation
Inoise = base line noise
Rpip = pipette resistance
Rtot = Total resistance
R1 Intial seal resistance (pre-setvalue)
R2 Seal resistance required for progression to whole cell

Vhold = Pre set holding potential in mV
Imon = current monitor output
i = pre-set holding current
Ihold base line holding current for holding potential
dpip = pipette holder module motor position
dpip0 = pipette holder module motor position start
dpip1 = pipette holder module motor position pipette on
pclamp = position of pipette clamp/loader encoder
pclamp = 0 pipette not clamped (loading position)
pclamp = 1 pipette clamped (recording position)
rotclamp = position of rotory stage mounting for pipette loader
rotclamp = 0 recording position
rotclamp = 1 pipette filling position
pipfil = pipette filler position
pipfil = 0 pre-/post-fill position
pipfil = 1 fill position
pipsyringe = pipette filler driven syringe position
pipsyringe = dv driven syringe movement required to fill pipette (pre-set value)
cclamp = position of capillary clamp/loader encoder
cclamp = 0 capillary not clamped (loading position)
cclamp = 1 capillary clamped (recording position)
pload = pipette loader empty signal
pload = 0 pipettes in loader
pload = 1 pipette loader empty
cload = capillary loader empty signal
cload = 0 capillaries in loader
cload = 1 capillary loader empty
celldiph = horizontal translator for cell dip
celldiph = 0 cell storage encoder position (pre-set)
celldiph = 1 dip encoder position (pre-set)
celldipv = verticle translator for cell dip
celldipv = 0 pre-/post- dip encoder position (pre-set)
celldipv = 1 capillary dip encoder position (pre-set)
tdelay = variable delay between clamping capillary and starting to patch clamp
dt = time interval
dt1 = pre-set waiting time interval suction off (s)
dt2 = pre-set suction time interval (s)
dt3 = pre-set suction time interval (s)
dt4 = pre-set suction time interval (s)
dt5 = pre-set suction time interval (s)
x = suction increment factor
f = frequency of seal test pulse
detectmin = 0 -ve capacitance transient (3Inoise threshold) not detected
detectmin = 1 -ve capacitance transient (3Inoise threshold) detected
detectmax = 0 +ve capacitance transient (3Inoise threshold) not detected
detectmax = 1 +ve capacitance transient(3Inoise threshold) detected

I = Whole cell mode flag
I = 0 Not whole cell
I = 1 Whole cell mode established
singlemV = pre-set voltage test pulse
curr = current recorded between pre-set cursors during voltage step
testcurr = pre-set value for current required to start experimental protocol
Valve1 – 8 = solenoid valves controlling supply of solution to perfusion dish
tv = time interval for valve activation (pre-set)
drain valve = controls suction supply to perfusion dish

Control Logic

Control logic according to a further embodiment is illustrated as a flow diagram in figure 8. Exemplary logic steps within each of the functions shown in the flow diagram are set out below.

00 Initialisation

d = d0
dpip = dpip0
pclamp = 0
pipfil = 0
rotclamp = 0
cclamp = 0
celldiph = 0
dapp = dapp0
Rtot >/= 20M
Imon = Inoise
P = 0
If pload = 0 and cload = 0 then GOTO 01
If pload = 1 then report "Re-load pipette cassette" and GOTO 19
If cload = 1 then report "Re-load capillary cassette" and GOTO 19

01 Autofeed

Move capillary clamp motor cclamp = 1
Move celldiph translator to celldiph =1
Move celldipv translator to celldipv =1
Move celldipv translator to celldipv=0
Move celldiph translator to celldiph = 0
start timer
wait for variable delay = tdelay
GOTO 02

02 Pipette load/fill

Move pipette clamp motor pclamp = 1
Move rotation stage 180 degrees rotclamp = 1
move pipfil until pipfil = 1
move motor driven syringe drive until pipsyringe = dv
move pipfil until pipfil = 0
move rotation stage 180 degrees rotclamp = 0
move dpip = dpip1
GOTO 03

03 Junction null

Seal test signal on
Compensate Cfast
Move patch module down until Imon >/< Inoise
Record patch module motor position d=d1
Activate Junction null
Measure Rtot
Rtot=Rpip
If Rpip <10M and >/=4M GOTO 04 else Report "Pipette resistance out of range" and GOTO 20

04 Formation of Gseal

Measure Rtot
If Rtot=/> 2Rpip
Suction on P=-pmmHg
Move patch module down until d=d2
(d2 =pre-set recording position)
GOTO 05

04.1
If Rtot <2 Rpip
Wait for time t1
After time t1 move patch module upwards until Rtot>2Rpip then stop. Wait until

Rtot=2Rpip then move patch module down until d=dl

If d=dl and Rtot=/> 2Rpip then suction on P = -pmmHg
and move patch motor module down until d=d2, else GOTO 04.1 until maximum of 5 iterations, then GOTO 20

05 Seal test loop

05.1 N=N+1

Measure Rtot with -pmmHg for time interval delta t2
If Rtot >/= R1 and dt </= dt2
Then suction off P = P0 until Rtot =/> R2 or dt=t1

If Rtot < R1 and dt >t1
Then repeat 05.1 until N=5 or Rtot =/> R2

If N = 5 and Rtot < R2 Then suction on –xpmmHg
Repeat 05.1 until N = 5 or Rtot =/> R2

If N = 5 and Rtot < R2 Then suction on –xpmmHg
Repeat 05.1until N = 5 or Rtot =/> R2 or x = pmax

If x=pmax and Rtot < R2 Then Report "Unable to obtain G seal" GOTO20

If Rtot =/> R2 then GOTO 06 for Whole Cell Mode or GOTO 08 for Cell Attached Patch Mode

06 Whole Cell - Threshold Method

Compensate Cfast
I = 0
hp = vhold
06.1 Suction on –pmmHg until I=1 or dt= dt3
06.2 Transient detection
N = N+1

If detectmax= 1 and detectmin = 1
Then I = 1
Repeat 06.2 until detectmax = 0 and detectmin = 0 or N= 10

If N = 10 Then GOTO 07

If detctmax = 0 and detectmin = 0
Then GOTO 06.1

07 Cell quality test

Measure Rs and Ihold
If Rs =/> 3Rpip Suction on –pmmHg and start timer

When Rs < 3Rpip suction off

If Ihold < ipA then and Rs < 3Rpip then seal test signal off and GOTO 08
If Ihold >ipA and time int = dt4 then GOTO 20

If Rs =/> 3Rpip and time int = dt5 then suction on –pmmHg

If Rs < 3Rpip and Ihold < ipA then seal test signal off and GOTO 08

08 Experimental protocol

Apply single voltage step to singlemV
Measure current amp during voltage steps
If curr < testcurr then stop voltage steps
Report "Control current out of range"
GOTO 15

If curr =/> testcurr activate voltage step protocol GOTO 09

Measure Rs
If Rs =/> 3Rpip stop voltage protocol and GOTO 07
Measure Ihold during interval between voltage steps

If Ihold > ipA then stop voltage step/drug application protocol and GOTO 01

09 Voltage step protocol

Uses program already available
Program must call drug application sub-routine 10

10 Drug/compound application

drain valve on
Fill drug application chamber Valve1 on for time interval tv

Move drug application micromanipulator down to d1 –dapp1 (NB –ve value represents downward movement)
Move dapp = d1 + dapp2 and d= d2 + dapp2 simultaneously
Move d = d3 glass capillary moved up
Call software controlling flow control valves

15 Reset Autopatch

GOTO 00.

19, 20 Steps 16 onwards are routines for setting up the apparatus controlled by the software/method and do not relate to the inventive operation of the apparatus and their design is within the normal competency of the skilled person. Steps 19 and 20 relate respectively to reloading the pipette and capillary cassettes and to checking and/or resetting of the apparatus if operation is unsuccessful.

Figure 9 is a flow diagram illustrating steps 03 and 04, which relate to formation of the G-seal. These steps comprise the most important advantageous steps in controlling the method and apparatus described herein.

In step 03 (junction null), the pipette tip is initially spaced below the meniscus at the end of the capillary. The logic, or software, then controls the patch module motor to move the pipette tip towards the meniscus until contact is made, detected by electrical contact therebetween. The movement is then stopped while the pipette resistance is measured and the motor position

recorded (d=d1, as shown in figures 1b and 3). If Rtot is outside a predetermined range, the experiment is aborted.

In step 04, Rtot is measured and if it is above a predetermined threshold, it is assumed that a cell is positioned on the pipette tip so suction is applied to the pipette and the logic controls the patch module motor to move the pipette tip further into the liquid within the capillary to a predetermined recording position (d=d2, as shown in figures 1b and 3). The logic then moves to step 05 to test the G-seal.

If at the start of step 04, Rtot is less than the predetermined threshold, the logic assumes that there is no cell at the pipette tip. The logic then waits for a predetermined time interval t1 before controlling the patch module motor to move the capillary away from the pipette until Rtot is measured to be greater than the predetermined threshold, when the movement is stopped. It is believed that in this position the pipette tip is still in contact with the liquid in the capillary but only via a neck, or bridge, of liquid drawn out by surface tension between the capillary and the pipette. The logic then waits until Rtot drops to equal the predetermined threshold. The logic then controls the patch module motor to return the pipette tip to d=d1, the position when it first contacted the capillary meniscus in step 03. If Rtot is then greater than the predetermined threshold it is assumed that contact with a cell has been made at the pipette tip, suction is applied to the pipette and the logic controls the patch module motor to move the pipette into the capillary to the predetermined recording position at d=d2.

It is believed that waiting for the time interval t1, which may be between 0.5 and 10 seconds, or preferably about 1 to 5 seconds, permits movement of the cells at the capillary tip, which is encouraged by the movement of the pipette tip to draw out the capillary meniscus.

If Rtot is still less than the predetermined threshold, the steps of waiting for time t1 and slightly moving the pipette are repeated for a predetermined number of iterations until a failure condition (step 20) is reached.

Claims

1. A method for providing a cell attached to the tip of a patch clamp pipette and having a high resistance (Giga Ohm) electrical seal between an area of the cell membrane and the tip, which includes the steps of:
i) providing a capillary tube containing a suspension of cells in a liquid;
ii) causing the formation of a layer of cells at one end of the capillary tube at the interface between the air and the liquid in which the cells are suspended;
iii) bringing the tip of the patch clamp pipette into contact with the interface by moving one or both of the pipette and the tube respectively together along a common axis of movement;
iv) contacting the tip with a cell in the cell layer at the interface; and
v) causing attachment of the cell to the tip.

2. A method according to claim 1 in which the liquid in which the cells are suspended is an extracellular physiological solution.

3. A method according to claim 1 in which the layer of cells is several cells deep and loosely packed.

4. A method according to claim 1 in which the layer of cells is formed by mounting the capillary tube in an essentially upright orientation and allowing the suspended cells to sediment to the downward end of the tube to collect there in a layer.

5. A method according to claim 1 in which the capillary tube is mounted essentially upright with the interface at a lower open end of the tube and the pipette is mounted essentially upright with the tip upwardly pointing.

6. A method according to claim 1 in which the capillary tube and pipette are concentrically mounted with the capillary tube in a fixed position and the pipette movable along the common axis.

7. A method according to claim 1 in which the capillary tube and pipette are concentrically mounted with the pipette in a fixed position and the capillary tube movable along the common axis.

8. A method according to claim 1 wherein gentle suction is applied to the pipette during contact with the interface end during the step of contacting the tip with a cell.

9. An apparatus for carrying out the method of any preceding claim which is a computer controlled apparatus including the following elements:
i) a patch clamp amplifier;
ii) a source of variable suction for a patch clamp pipette under the control of the patch clamp amplifier;
iii) a holder for a capillary tube to be mounted vertically;
iv) a holder for a patch clamp pipette to be mounted vertically in the same axis as the capillary tube in an inverted orientation with the tip pointing upwardly;

v) a manipulator for controlling relative movement of the capillary tube and pipette along a common axis of movement under feedback control from the patch clamp amplifier and allowing for the tip of the pipette to enter a downwardly facing end of the capillary tube.

10. An apparatus according to claim 9 which includes an array of a multiplicity of capillary tubes and an array of a multiplicity of pipettes.

11. A computer-program-controlled patch clamping process for carrying out the method of any of claims 1 to 8.

12. A computer-program-controlled patch clamping process for controlling the apparatus of claim 9 or 10.

13. A computer-readable medium having a program recorded thereon, where the program is to make the computer control the method of any of claims 1 to 8 or the apparatus of claim 9 or 10.

FIG 1 a

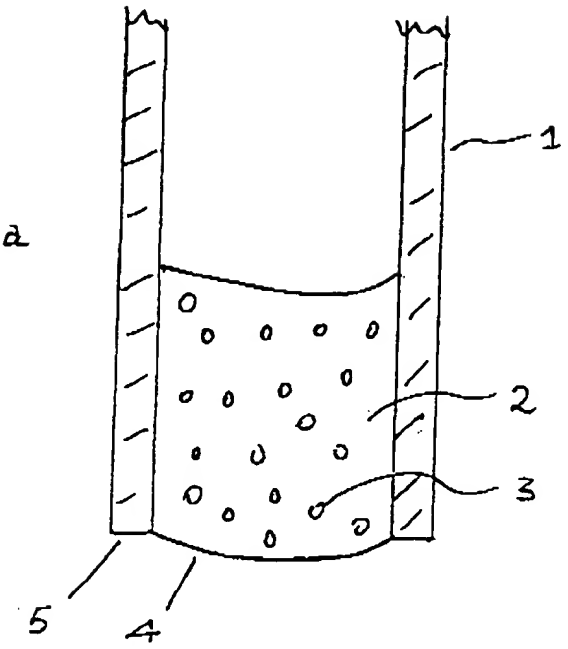


FIG 1 b

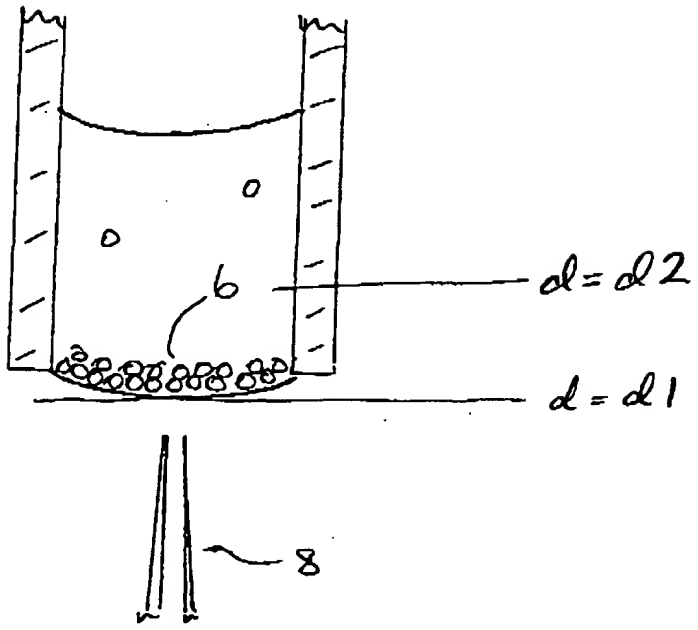
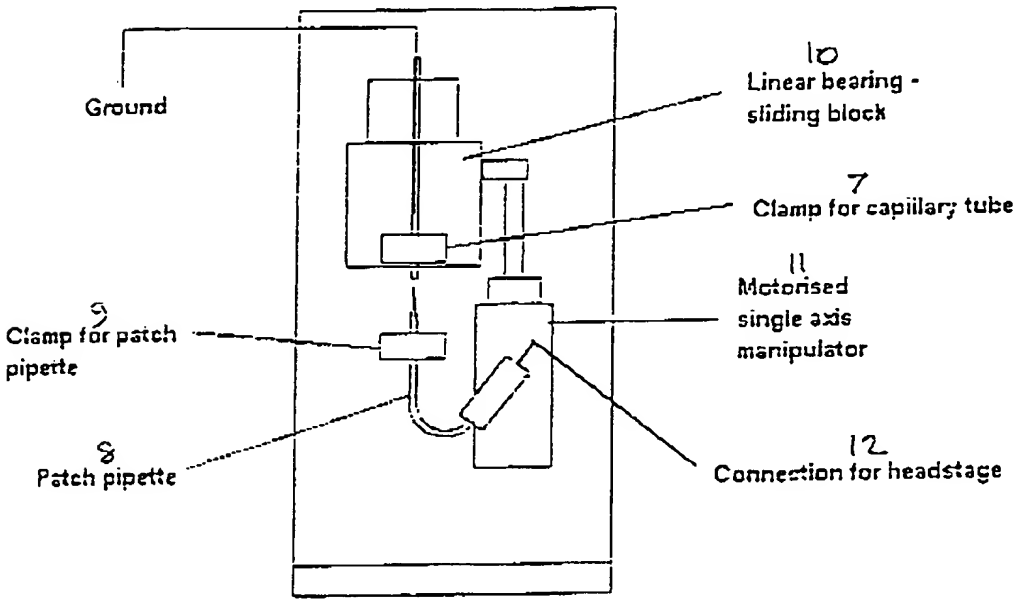


FIG 2


Ground
10
Linear bearing - sliding block
7
Clamp for capillary tube
11
Motorised single axis manipulator
9
Clamp for patch pipette
8
Patch pipette
12
Connection for headstage

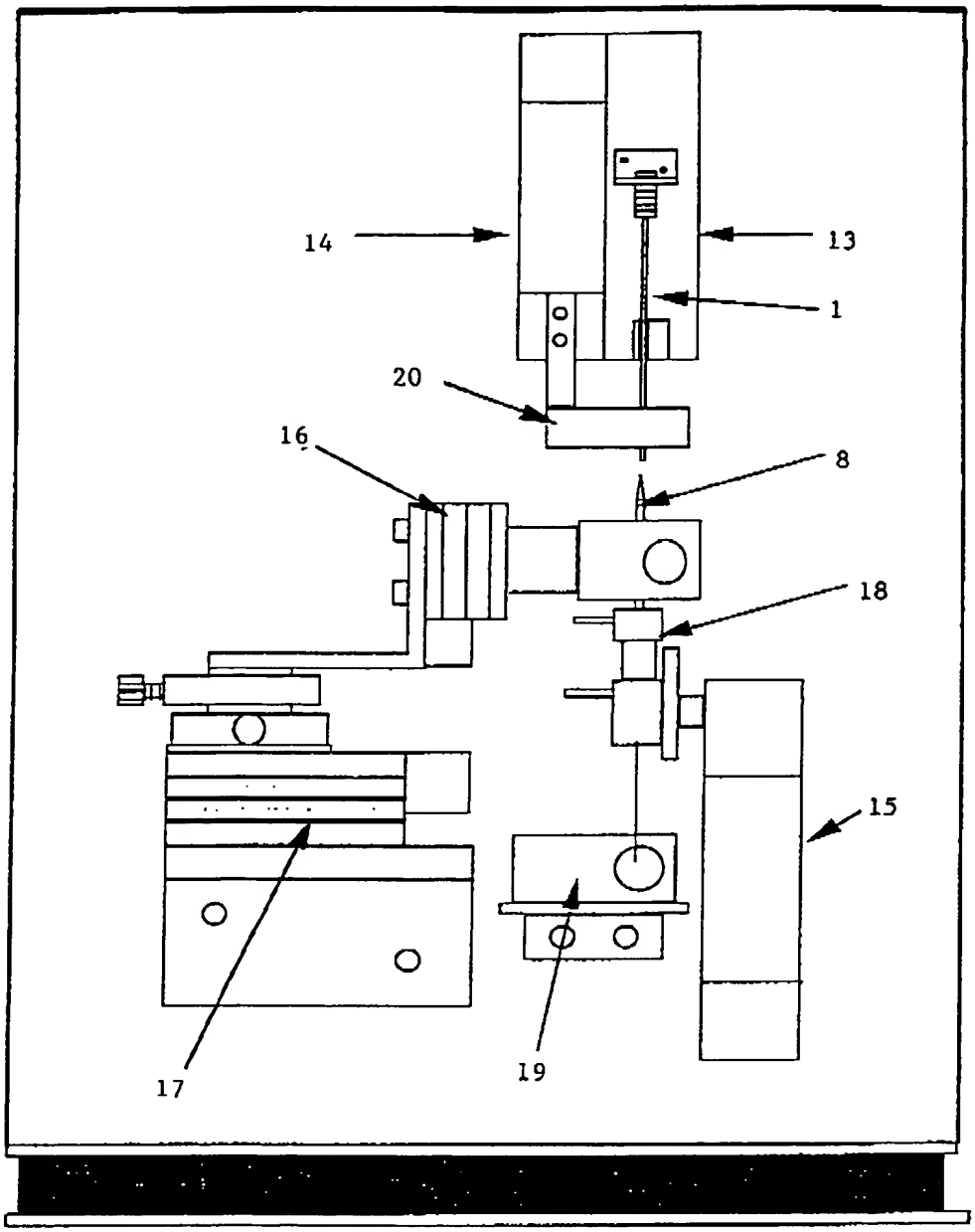
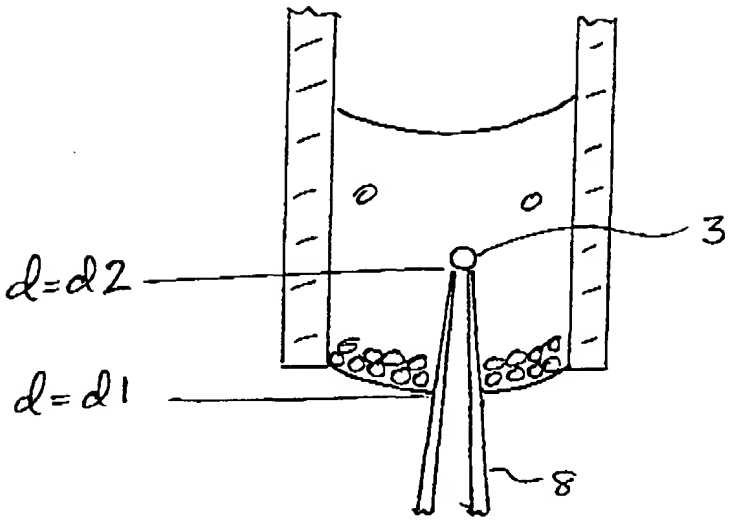


FIGURE 2a

FIG 3



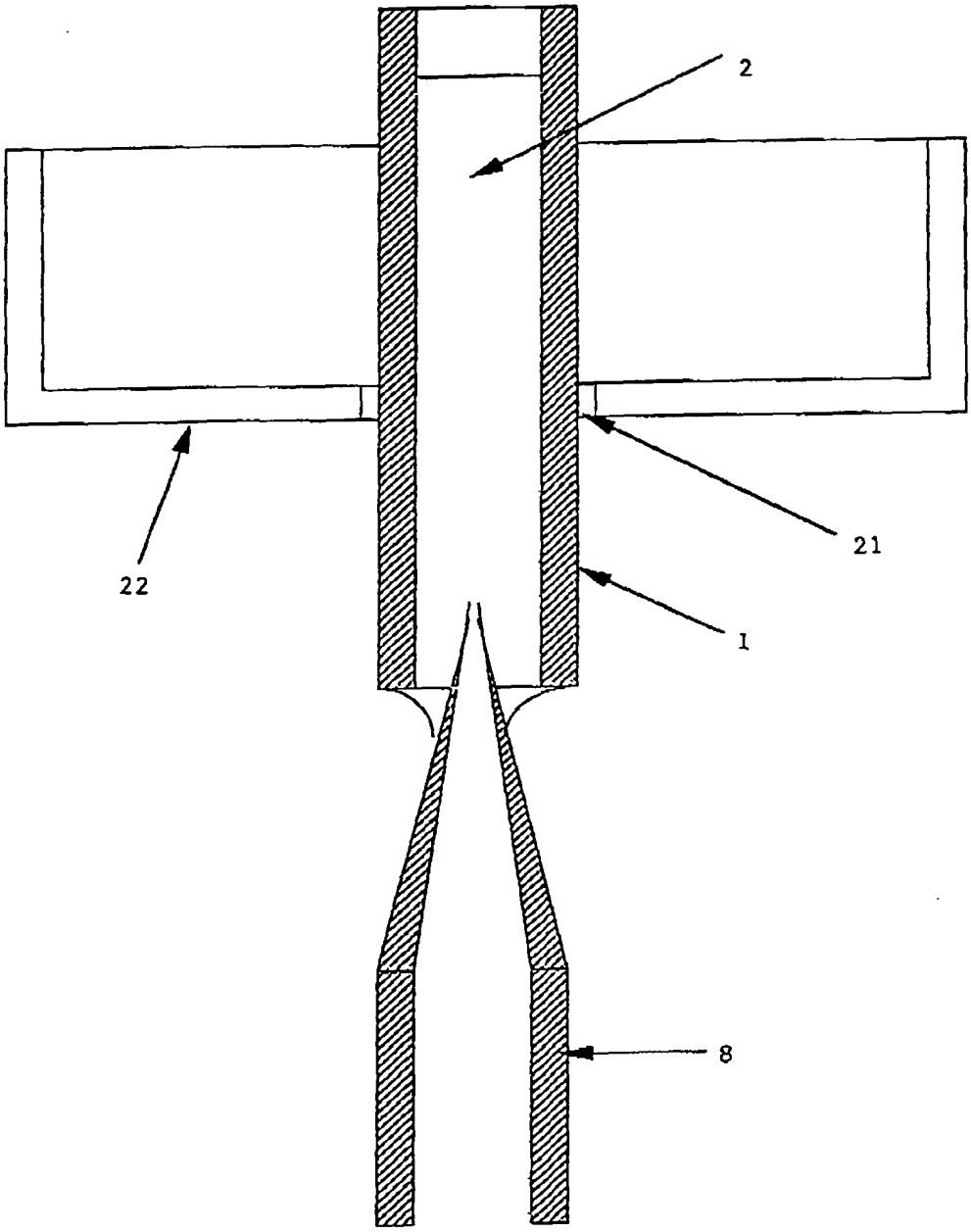


FIGURE 4

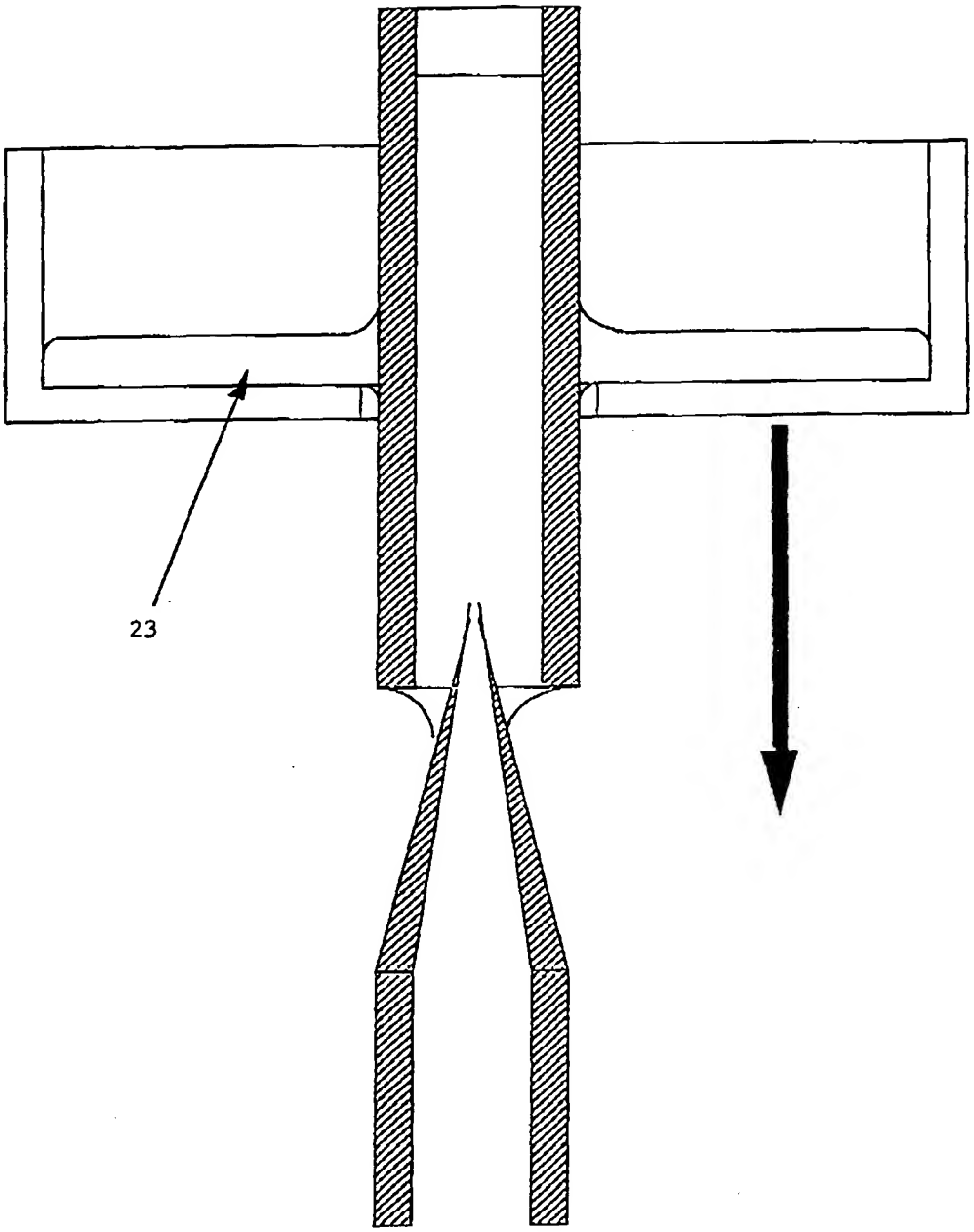


FIGURE 5

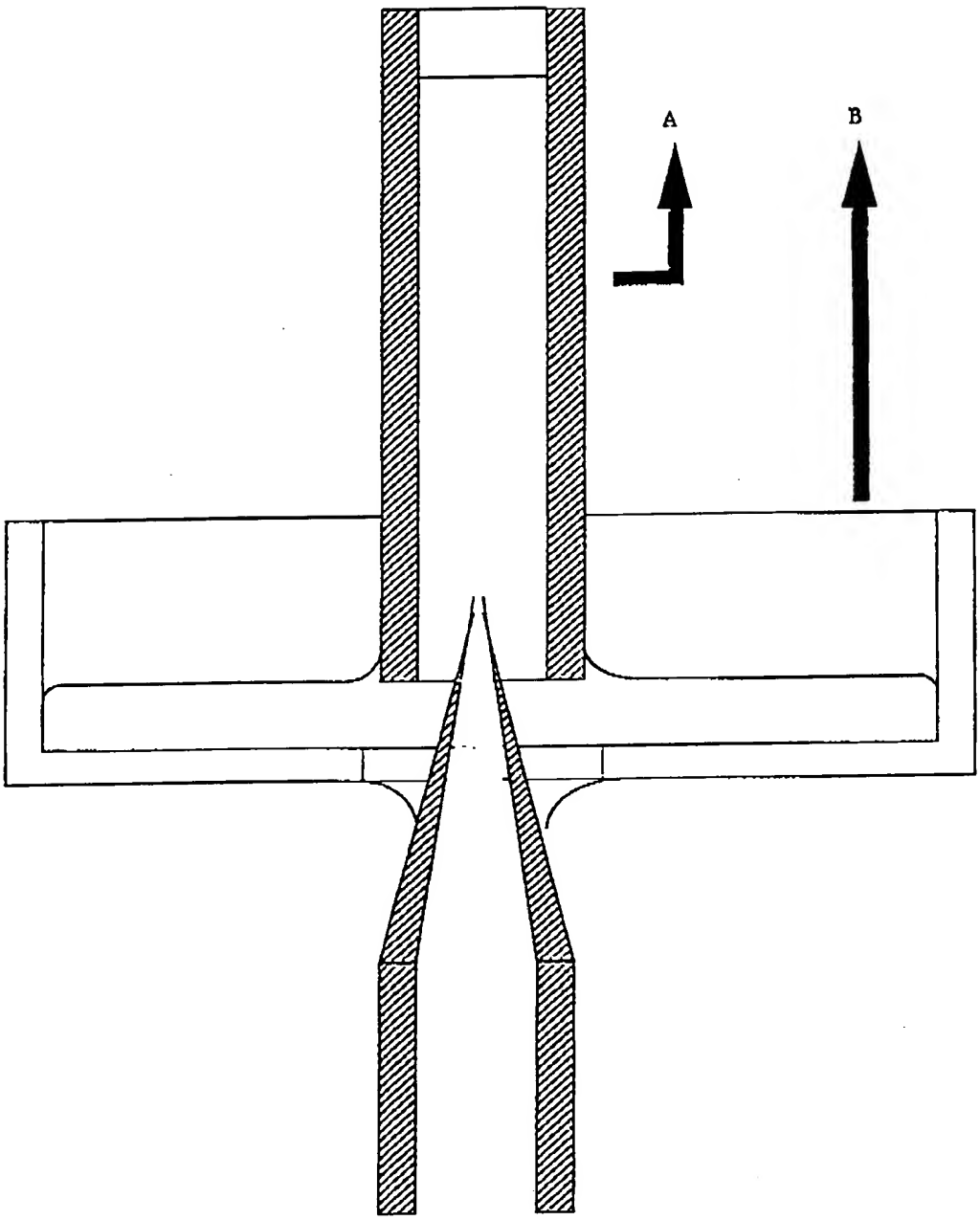


FIGURE 6

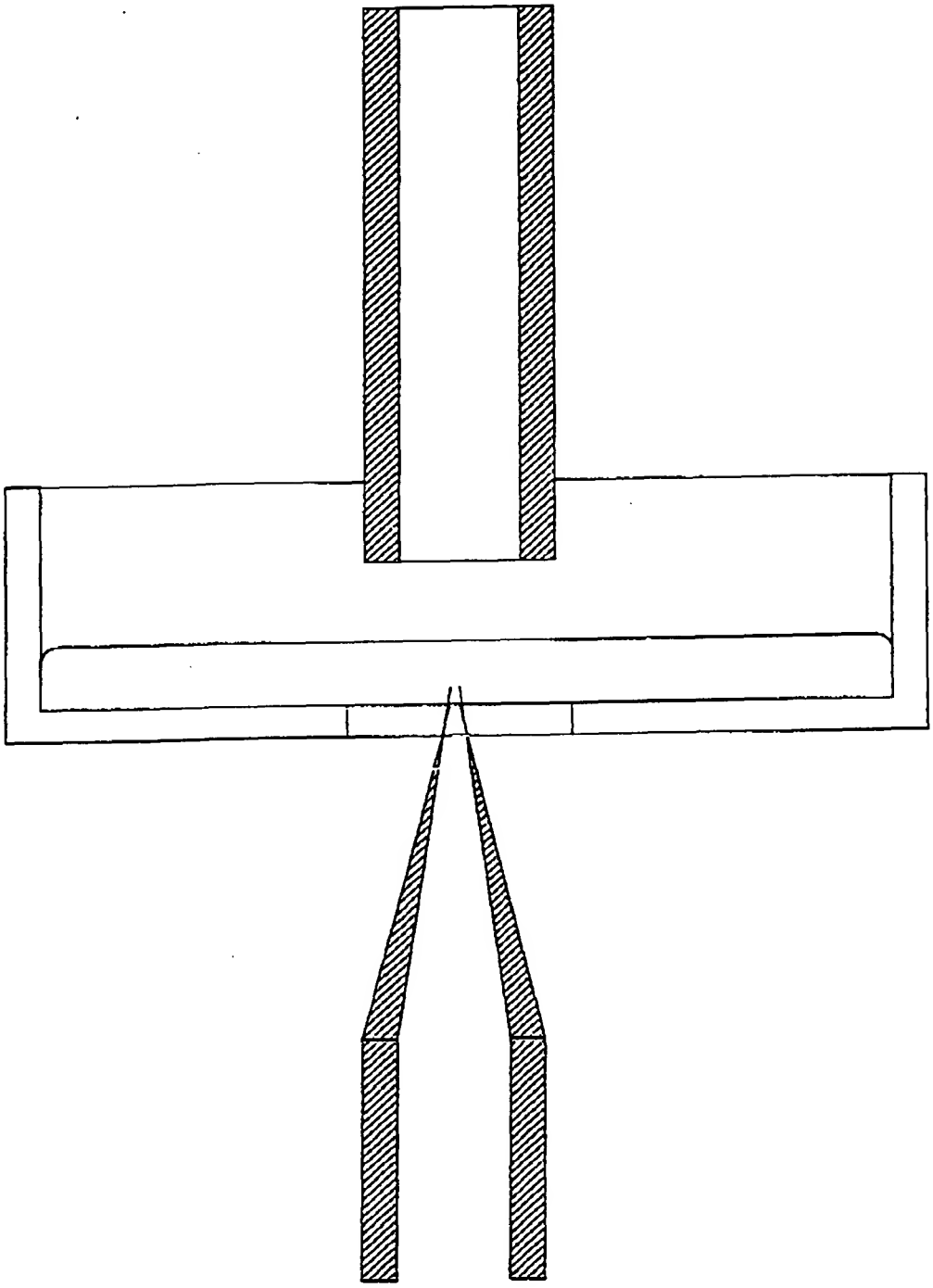


FIGURE 7

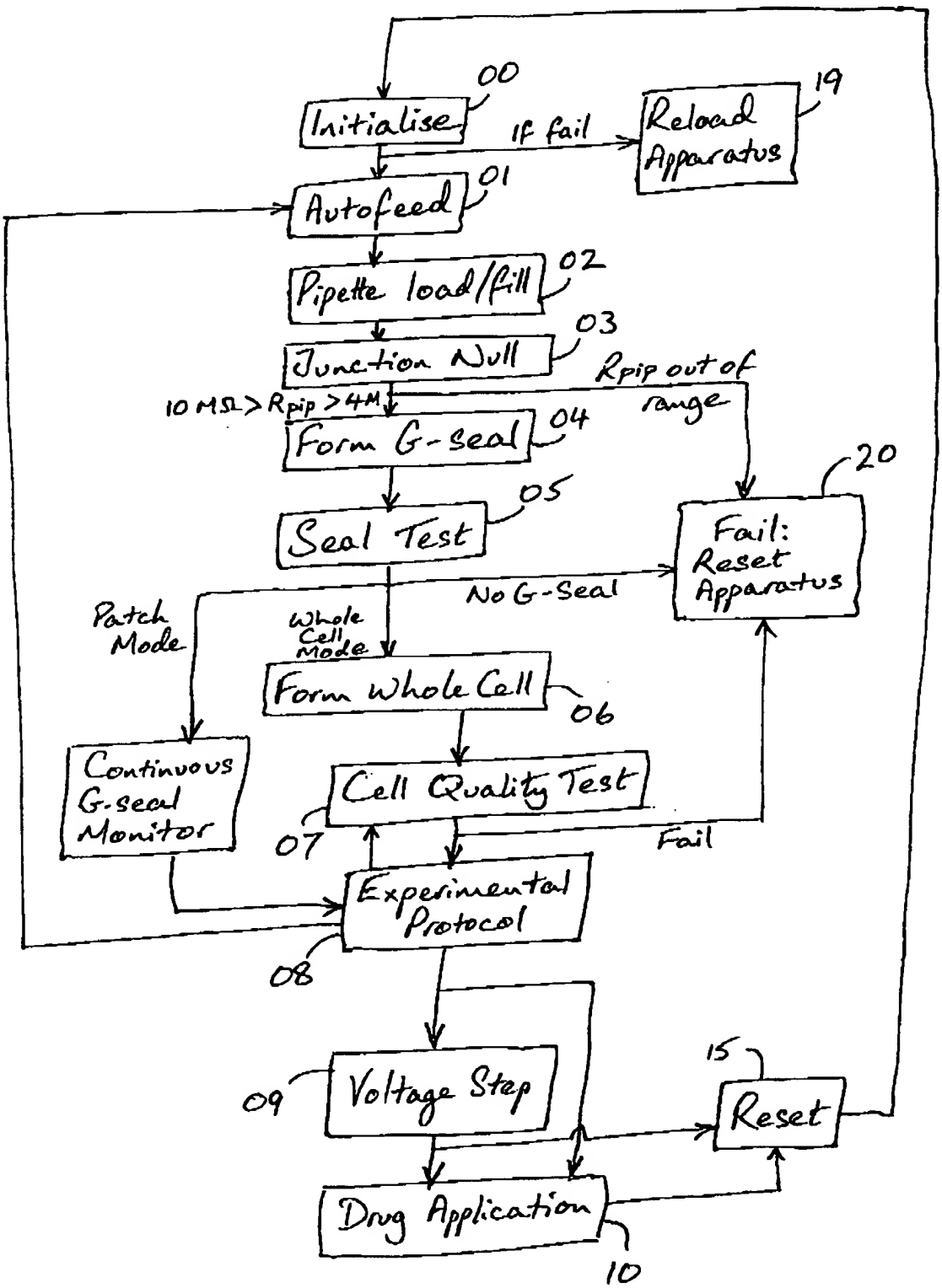

00
Initialise
If fail
19
Reload Apparatus
01
Autofeed
02
Pipette load/fill
03
Junction Null
Rpip out of range
10 MΩ > Rpip > 4M
04
Form G-seal
20
05
Seal Test
Fail: Reset Apparatus
No G-Seal
Patch Mode
Whole Cell Mode
Form Whole Cell
06
Continuous G-seal Monitor
Cell Quality Test
07
Fail
Experimental Protocol
08
09
Voltage Step
15
Reset
Drug Application
10

FIGURE 8

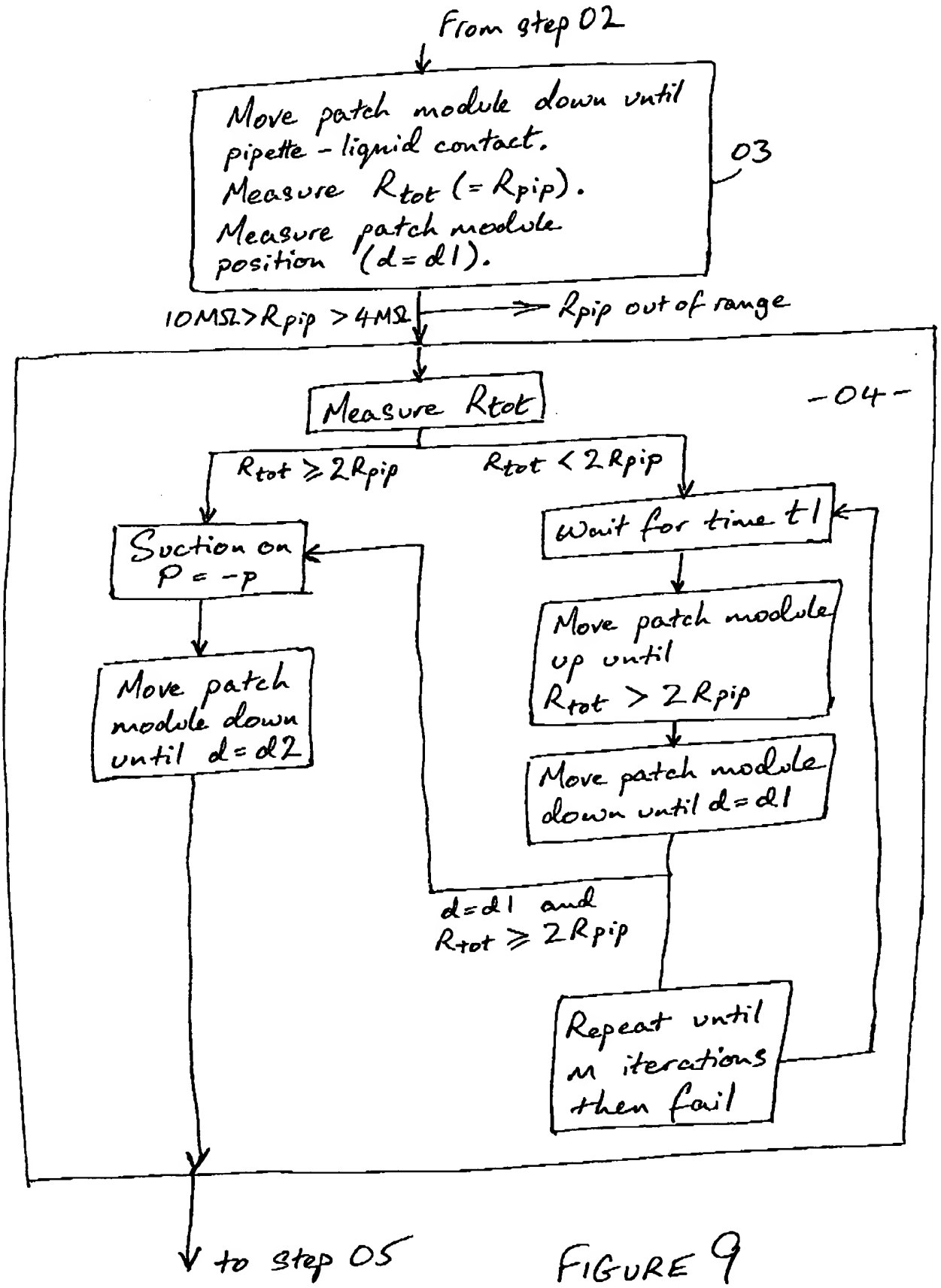

From step 02
Move patch module down until pipette - liquid contact.
Measure R_{tot} ($= R_{pip}$).
Measure patch module position ($d = d1$).
03
10MΩ > Rpip > 4MΩ
Rpip out of range
Measure Rtot
-04-
Rtot ≥ 2Rpip
Rtot < 2Rpip
Suction on P = -p
Wait for time t1
Move patch module up until Rtot > 2Rpip
Move patch module down until d = d2
Move patch module down until d = d1
d = d1 and Rtot ≥ 2Rpip
Repeat until M iterations then fail
to step 05

FIGURE 9

PCT/GB 99/04073

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